UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/672,484	09/25/2003	Roland Contreras	13748Z	8325

23389 7590 06/09/2009

SCULLY SCOTT MURPHY & PRESSER, PC
400 GARDEN CITY PLAZA
SUITE 300
GARDEN CITY, NY 11530

EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
06/09/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/672,484	CONTRERAS ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 March 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *74-90,92-95,97-107,109-112,114 and 115* is/are pending in the application.

4a) Of the above claim(s) *74-89* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *90, 92-95, 97-107, 109-112 and 114-115* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/16/09 has been entered.

Claims 74-90, 92-95, 97-107, 109-112 and 114-115 are pending in the present application.

Claims 74-89 were withdrawn previously from further consideration because they are directed to an invention nonelected with traverse in the reply filed on 8/9/06.

Accordingly amended claims 90, 92-95, 97-107, 109-112 and new claims 114-115 are examined on the merits herein.

Response to Amendment

All of the prior art rejections under 35 U.S.C. 102(e) and 35 U.S.C. 103(a) that were set forth in the Office action mailed on 9/16/08 were withdrawn in light of Applicant's amendment.

Written Description

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Amended claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. ***This is a new ground of rejection.***

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed " Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

The present invention is drawn to a genetically engineered strain of *Pichia* transformed with **a nucleotide sequence coding for a *T. reesei* α-1,2-mannosidase (including but not necessarily limited to a full length *T. reesei* α-1,2-mannosidase) or a functional part (fragment) thereof**, wherein the genomic OCH1 gene is said strain is disrupted and **as a result of the expression of said *T. reesei* α-1,2-mannosidase or said functional part, the genetically engineered strain produces $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure**; a kit comprising the same strain and a method of reducing glycosylation of an endogenous glycoprotein and/or a heterologous glycoprotein using the same strain.

Apart from disclosing the use of an expression vector encoding the full-length *T. reesei* α-1,2-mannosidase for transforming a *Pichia pastroris* strain whose genomic OCH1 gene is disrupted to attain a predominant N-glycan structure or a predominant intermediate N-glycan structure, the instant disclosure fails to provide sufficient written description for **a representative number of species for a broad genus of a functional part or a fragment of *T. reesei* α-1,2-mannosidase whose expression in the *Pichia* strain results in the specific desired result as broadly claimed**. The instant specification fails to describe **the essential core structure(s)/element(s) possessed by any functional part or any fragment of *T. reesei* α-1,2-mannosidase so that the functional fragment is sufficiently stable and active in the *Pichia* strain to yield the specific desired activity as claimed.** As defined by the instant specification, the term "functional part" is meant to be a polypeptide fragment of an α 1,2-mannosidase which substantially retains the enzymatic activity of the full-length protein (page 15, lines 15-17). At about the effective filing date of the present application (6/30/2000), the absolute enzymatic activity of *T. reesei* α-1,2-mannosidase **is not correlated** with the production of a predominant N-glycan structure or a predominant intermediate N-glycan structure as evidenced at least by the teachings of Martinet et al (Biotechnology Letters 20:1171-1177, 1998; IDS) and Callewaert et al. (FEBS Letters 503:173-178, 2001). Moreover, Choi et al (PNAS 100:5022-5027, 2003) also teach that **a proper length of the α-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains** (see at least page 5026, col. 1, second full paragraph).

The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants' filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc., 48 USPQ2d 1641, 1646 (1998). The skilled artisan cannot envision the detailed structure of **a representative number of species for a broad genus of a functional part or a fragment of *T. reesei* α-1,2-mannosidase whose expression in the *Pichia* strain results in the specific desired result as broadly claimed**, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention and reference to a method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Enablement

Amended claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A genetically engineered strain of *Pichia*, wherein said strain is transformed with **a nucleotide sequence coding for a full-length *T. reesei* α-1,2-mannosidase**, wherein said *T. reesei* α-1,2-mannosidase is genetically engineered to contain an ER-retention signal and the genomic Och1 gene in said strain is disrupted such that said strain fails to produce a functional Och1 protein, and wherein as a result of expression of said *T. reesei* α-1,2-mannosidase said strain produces $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structrure; a kit comprising the same strain and a method of reducing the glycosylation of an endogenous glycoprotein and/or a heterologous glycoprotein expressed in the same genetically engineered *Pichia* strain;

does not reasonably provide enablement for **a genetically engineered strain of *Pichia* transformed with other nucleotide sequences coding for a *T. reesei* α-1,2-mannosidase or functional part thereof to attain the specific desired result, a kit and a method of using the same strain as broadly claimed**. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The present disclosure is not enabled for the instant broadly claimed invention for the reasons discussed below.

1. The breadth of the claims

The instant claims are directed to a genetically engineered strain of *Pichia* transformed with **a nucleotide sequence coding for a *T. reesei* α-1,2-mannosidase (including but not necessarily limited to a full length *T. reesei* α-1,2-mannosidase) or a functional part (fragment) thereof**, wherein the genomic OCH1 gene is said strain is disrupted and **as a result of the expression of said *T. reesei* α-1,2-mannosidase or said functional part, the genetically engineered strain produces $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure**; a kit comprising the same strain and a method of reducing glycosylation of an endogenous glycoprotein and/or a heterologous glycoprotein using the same strain.

2. The state and the unpredictability of the prior art

At about the effective filing date of the present application (6/30/2000), little was known about a modification of the protein glycosylation pathway in a *Pichia* yeast strain to generate Man5GlcNAc2 as a predominant N-glycan structure or a predominant intermediate N-glycan structure as evidenced at least by the teachings of Martinet et al (Biotechnology Letters 20:1171-1177, 1998; IDS) and Callewaert et al. (FEBS Letters 503:173-178, 2001). In contrast, there are several known double and triple mutants of *Saccharomyces cerevisiae* that have been characterized to produce $Man_5GlcNAc_2$ and/or $Man_8GlcNAc_2$ as a predominant glycoform species (Nakanishi-Shindo et al, J. Biol. Chem. 268:26338-26346, 1993; IDS; and Chiba et al, J. Biol. Chem. 41:26298-26304, 1998; IDS). Additionally, *T. reesei* α-1,2-mannosidase was overexpressed in a *pichia pastoris* strain, however the expression and/or activity of *T. reesei* α-1,2-

mannosidase was not sufficient to generate $Man_5GlcNAc_2$ as the predominant glycoform species (see Martinet et al and Callewaert et al references cited above). Several years after the effective filing date of the present application, Choi et al (PNAS 100:5022-5027, 2003) disclose that **a proper length of the α-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains** (see at least page 5026, col. 1, second full paragraph). Moreover, as is well recognized in the art any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. There is a high degree of unpredictability associated with the use of the claimed embodiment as evidenced by the teachings of Rudinger in discussing peptide hormones. Rudinger stated that "The significance of particular amino acids and sequences for different aspects of biological activity (for this instance the enzymatic activities of I-1,2-mannosidase or glucosidase II) can not be predicted a priori but must be determined from case to case by painstaking experimental study (Page 6, first sentence of Conclusions *In* J.A. Parsons, ed. "Peptide hormones", University Park Press, 1976; IDS). Furthermore, it should be further emphasized that the relationship between the sequence of a peptide and its tertiary structure associated for its activity is not well understood and is not predictable (Ngo et al., *In* Merz et al., ed. "The protein folding problem and tertiary structure prediction", Birkhauser, 1994; IDS). Since the prior art at the effective filing date of the present application does not provide any guidance regarding to the aforementioned issues, it is

incumbent upon the instant specification to do so. Furthermore, the physiological art is also recognized as unpredictable (MPEP 2164.03).

3. The amount of direction or guidance provided

Apart from disclosing the use of an expression vector encoding the full-length *T. reesei* α-1,2-mannosidase for transforming a *Pichia pastroris* strain whose genomic OCH1 gene is disrupted to attain a predominant N-glycan structure or a predominant intermediate N-glycan structure (see at least examples 2-3 and Figures 6-7 and 10), the instant specification fails to provide sufficient guidance (exemplification is part of a guidance) for a skilled artisan on how to make and use any encoded fragment or functional part of a *T. reesei* α-1,2-mannosidase for producing Man5GlcNAc2 as a predominant N-glycan structure or a predominant intermediate N-glycan structure. In addition to a high degree of unpredictability associated with the make and/or use of an enzyme fragment having a specific desired property as discussed above, it should be noted that full-length *T. reesei* α-1,2-mannosidase has a pH optimum of 5.0 while most enzymes active in the ER and Golgi apparatus of yeasts have pH optima that are between 6,5 and 7.5 (Gerngross, US 2002/0137134, IDS; see at least paragraph 68), it is unclear whether any fragment of the *T. reesei* α-1,2-mannosidase would still be stable and still sufficient active in the less than optimal environment of a yeast's ER to yield Man5GlcNAc2 as a predominant N-glycan structure or a predominant intermediate N-glycan structure. As already noted above, several years after the effective filing date of the present application, Choi et al (PNAS 100:5022-5027, 2003) disclose that **a proper length of the α-1,2-mannosidase catalytic domain is one of several factors**

that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains (see at least page 5026, col. 1, second full paragraph).

As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues set forth above, the breadth of the claims, and the state and the unpredictability of the relevant art, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Amended claims 90, 92-95, 104-107 and 109-112 are rejected under 35 U.S.C. 103(a) as being unpatentable over Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) in view of JP 8336387 (12/24/96; IDS), Nakanishi-Shindo et al. (J. Biol. Chem. 268:26338-26346, 1993; IDS) and Chiba et al. (J. Biol. Chem. 41:26298-26304, 1998; IDS). ***This is a new ground of rejection necessitated by Applicant's amendment.***

Martinet et al. teach the preparation of **plasmids for expression of full-length *T. reesei* α-1,2-mannosidase or a chimeric *S. cerevisiae/ T. reesei* α-1,2-mannosidase (a fusion of the catalytic domain of *T. reesei* α-1,2-mannosidase to the ER retention signal of *S. cerevisiae* MNS1) in *Pichia pastoris* strains GSIV-HAs and GSIVNAf1s derived from the parental strain GS115** (see Materials and Methods, particularly sections "Strains and culture conditions" and "Construction of plasmids for expression of *T. reesei* α-1,2-mannosidase in *P. patoris*"). Martinet et al. further teach that in all expression plasmids are derived from the pPICZB vector, and the *T. reesei* α-1,2-mannosidase gene was under transcriptional control of the AOX1 promoter (page 1172, col. 2, first full paragraph). Martinet et al. also disclose that **co-expression of heterologous *T. reesei* α-1,2-mannosidase in GSIVNAf1s resulting in partial trimming of the large influenza neuramidase (NA) N-glycans** (>Man14GlcNac2)

(see section "In vivo trimming of N-glycans by heterologous *T. reesei* α-1,2-mannosidase", and Figures 2A, 3). The **co-expression of the chimeric MNS1/*T. reesei* α-1,2-mannosidase in GSIV-HAs resulted in the formation of both trimmed and hyperglycosylation glycan products of hemagglutinin (HA)** (see page 1175, col. 2 and Fig. 4). Additionally, Martinet et al. note that **hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues** (page 1176, col. 1); and the results from the co-expression of the chimeric MNS1/*T. reesei* α-1,2-mannosidase in GSIV-Has **suggest that removal of mannose residues creates more ideal substrates for *P. pastoris* mannosyltransferrases, leading to elongation of truncated glycosyl chains, and not to complete α-1,2-mannosidase digestion** (page 1175, bottom of col. 2).

Within the scope of enablement, Martinet et al. do not teach the preparation of a genetically engineered strain of *Pichia*, wherein said strain is transformed with a nucleotide sequence coding for a full-length *T. reesei* α-1,2-mannosidase, wherein said *T. reesei* α-1,2-mannosidase is genetically engineered to contain an ER-retention signal and the genomic Och1 gene in said strain is disrupted such that said strain fails to produce a functional Och1 protein, and wherein as a result of expression of said *T. reesei* α-1,2-mannosidase said strain produces $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structrure; a kit comprising the same strain and a method of reducing the glycosylation of an endogenous glycoprotein

or a heterologous glycoprotein expressed in the same genetically engineered *Pichia* strain.

However, at the effective filing date of the present application, JP 8-336387 already taught the preparation of a vector construct comprising a portion of *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 in a *Pichia* yeast strain, including the GTS 115 (NRRL Y-15851) strain for inhibiting the elongation of sugar chains on glycoproteins for production of a glycoprotein having a sugar chain identical or similar to that of a medically useful biologically active protein (see at least the abstract in English, Fig. 9 on page 635 as well as col. 15, paragraph 33). The formation of $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structrure in the genetically modified Pichia yeast strain is absent in JP 8-336387.

Additionally, at the effective filing date of the present application Nakanishi-Shindo et al already taught that single, double and triple mutant strains of *Saccharomyces cerevisidae*, all of which contain the disruption of och1 gene; and they taught that analysis of PA oligosccharides from the och-1 mutant, och1 /nn1 mutant and **och1/mnn1/alg3 mutant** revealed that $Man_9GlcNAc_2$, $Man_8GlcNAc_2$ and a mixture of **$Man_5GlcNAc_2$ and $Man_8GlcNAc_2$** as a predominant species in these respective yeast mutant strains (see at least the abstract ; section entitled "Comparison of Invertase mobility between the Mutants" and particularly Figure 2).

Furthermore, Chiba et al. already taught the preparation of an expression vector encoding ER targeted HDEL-tagged full-length *Aspergillus saitoi* α-1,2-mannosidase for

expression in various *Saccharomyces cerevisiae* strains, including the **triple Δoch1Δmnn1Δmnn4 mutant strain**, and structural analysis of CBT sugar chains revealed that **the introduced α-1,2-mannosidase digested the sugar chains up to $Man_5GlcNAc_2$ and Figure 6b revealed that it is a predominant digestion product** (see abstract and the section "DNA constructs"; page26303, col. 1, third paragraph continues to col. 2 and Figure 6). Although a much lower molar ratio of $Man_5GlcNAc_2$ was found in the analysis of mannoproteins (Figure 6c), Chiba et al attributed this observation might be because of the difference in cell harvesting period (mannoproteins were recovered at mid-log phase of the culture instead of the recovery of CPY was done at stationary phase) and/or that the expression level of the introduced α-1,2-mannosidase might not be sufficient for complete trimming of each sugar chain and suggested the use of inducible promoters for its expression (page 26302, col. 1, last paragraph continues to first paragraph of col. 2). Furthermore, Chiba et al. disclosed that the **$Man_5GlcNAc_2$-oligosaccharide is the intermediate for the production of hybrid-type and complex-type sugar chains, the latter of which is better suited and more effective for the production of human therapeutics**.

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to modify the teachings of Martinet et al. by reducing glycosylation of a heterologous glycoprotein expressed in a *Pichia* strain by utilizing at least a *Pichia* strain whose genome comprising the disruption of the Och1/Mnn1, Och1/Mnn1/alg3 or Och1/Mnn1/Mnn4 genes together with the introduced ER targeted HDEL-tagged full-

length *T. reesei* α-1,2-mannosidase in light of the teachings of JP 8336387, Nakanishi-Shindo et al and Chiba et al discussed above.

An ordinary skilled artisan would have been motivated to carry out the above modification because the elimination or disruption of endogenous Och1/Mnn1, Och1/Mnn1/alg3 or Och1/Mnn1/Mnn4 genes in the genome of a yeast strain has been shown to facilitate a predominant formation of **$Man_5GlcNAc_2$ and/or $Man_8GlcNAc_2$**, with the latter being further trimmed by the action of an α-1,2-mannosidase targeted to ER as taught by Nakanishi-Shindo et al and Chiba et al. Moreover, ΔOCH1 *Pichia* yeast strain has been generated and formation of smaller and homogenous oligosaccharides in heterologous glycoproteins in the yeast strain has been attained as taught by JP 8-336387. Furthermore, Martinet et al. already noted at least that hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues (page 1176, col. 1).

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Martinet et al., JP 8-336387, Nakanishi-Shindo et al and Chiba et al., coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

In light of the Supreme Court Decision in KSR International Co. v. Teleflex Inc., 550 U.S.—82 USPQ2d 1385 (2007), the following rejection is applied.

Amended claims 97-106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) in view of JP 8336387 (12/24/96; IDS), Nakanishi-Shindo et al. (J. Biol. Chem. 268:26338-26346, 1993; IDS) and Chiba et al. (J. Biol. Chem. 41:26298-26304, 1998; IDS) as applied to claims 90, 92-95, 104-107 and 109-112 above, and further in view of Trombetta et al. (J. Biol. Chem. 271:27509-27516, 1996; IDS) ***This is a new ground of rejection.***

The combined teachings of Martinet et al, JP 8336387, Nakanishi-Shindo et al and Chiba et al. were already discussed above. However, none of the cited references teaches specifically that the genetically modified yeast *Pichia* further transformed with a vector comprising a nucleotide sequence coding for a glucosidase II or a functional part thereof.

However, at the effective filing date of the present application Trombetta et al. already disclosed cDNA sequences encoding endoplasmic reticulum glucosidase II derived from various sources; including a glucosidase II gene from *S.cerevisiae* (see at least the abstract and sections titled "Primary sequence of αSubunit" and "Identification of the *S. cerevisiae* functional homologue of mammalian glucosidase II catalytic subunit (α)" on pages 27511-27513). Trombetta et al further demonstrated that *S.cerevisiae* functional homologue of mammalian glucosidase II catalytic subunit α removes two α-

1,3-linked Glc units after removal of the terminal α-1,2-linked Glc residue in the core oligosaccharide $Glc_3Man_9GlcNAc2$ (page 17514, col. 1, second paragraph).

It would also have been obvious and within the scope of skill for an ordinary artisan to further modify the combined teachings of Marinet et al, JP 8336387, Nakanishi-Shindo et al and Chiba et al. by also further transforming the genetically engineered strain expressing an exogenous ER-targeted glucosidase II in light of the teachings of Trombetta et al discussed above.

An ordinary skilled artisan would have been motivated to further carry out the above modification because expressing an exogenous and ER targeted glucosidase II in the genetically engineered *Pichia* strain would further enhance the removal of any unprocessing glucose residues and further ensuring the formation $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structrure in the yeast strain. Particularly, Martinet et al. already suggested that **unprocessed glucose residues or capping glucose residues may block α-1,2-mannosidase treatment, and that these may be responsible for incomplete trimming of NA oligosaccharides to Man5GlcNAc2** (page 1175, col. 2, first paragraph; page 1176, col.1, bottom of the second paragraph).

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modifications in light of the teachings of Martinet et al., JP 8-336387, Nakanishi-Shindo et al., Chiba et al., and Trombetta et al.; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related in part to the above new grounds of rejection in the Amendment filed on 3/16/09 (pages 13-18) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue basically that Martinet et al primary references teaches the preparation of two forms of *T. reesei* α-1,2-mannosidase: a secreted and an intracellular form, and with respect to the results obtained for the secreted form the expression of the ER targeted fusion chimeric form resulted in worse glycosylation with more higher mannose forms being formed for heterologous proteins. Accordingly, an ordinary skilled artisan would not have been motivated to select the portion of the reference relating to the intracellular expression of *T. reesei* α-1,2-mannosidase to combine with the teachings of JP 8-336387, or a reasonable expectation of success in significantly reducing hyperglycosylation and/or producing Man5GlcNAc2 as a predominant glycoform or a predominant intermediate glycoform. Applicants also argue that the JP 8-336387 does not show the production of any Man8 glycoforms, much less Man8 as a predominant glycoform which could be converted to Man5 by an α-1,2-mannosidase. Applicants further argue that the prior art teaches significant unpredictability as evidenced by the teachings of Nakanishi-Shindo et al (Exhibit 2) that showed that a double mutant of *och1 mnn1* was required to obtain Man8 as a predominant glycoform

in *S. cerevisiae*, whereas a och1 single mutant produced a mixture of Man8, Man9, and Man10 N-glycan with Man9 being the dominant form; the teachings of Chiba et al (Exhibit 3) reported obtaining Man5GlcNAc2 in *S. cerevisiae* using a och1 mnn1 mnn4 triple mutant and an α-1,2-mannosidase; the enzyme encoded by Mnn4 results in mannosylphosphorylation of the baker yeast's N-glycan, a modification which was known to be present in 30% of N-glycans in *Pichia* as shown by Grinna et al (Exhibit 4). Accordingly, **those skilled in the art would not have reasonably expected that a disruption of the Och1 gene alone would have led to a production of Man8GlcNAc2 in an effective amount to permit the subsequent production of Man5GlcNAc2 as a predominant glycoform**. Finally, Applicants also argue unreasonable expectation of success since inactivation of the Och1 gene in *S.cerevisiae* was severely detrimental to cell viability as shown in Exhibit 5 (Nagasu et al reference) and the statement commented by the post-filing art in the form of the Vervecken et al reference in Exhibit 6 that "N-glycan homogenization in baker yeast has been an inefficient process, and the strains obtained might be too weak for general use in glycan engineering".

Firstly, it should be noted that apart from **claims 114-115 which were not rejected by the above new grounds of 103 rejections**, the rejected claims encompass the make and/or use of **a genetically engineered strain of *Pichia* whose genome comprises disruption of genes such as Och1/Mnn1, Och1/Mnn1/alg3 or Och1/Mnn1/Mnn4, and not necessarily limited only the a single disruption of the och1 gene in the genome of a *Pichia* strain.**

Secondly, **please refer to the above new grounds of rejections particularly the teachings of Nakanishi-Shindo et al., and Chiba et al** with respect to the issue of expectation of success for obtaining Man5GlcNAc2 as a predominant N-glycan structure or a predominant intermediate N-glycan structure in a genetically engineered *Pichia* strain.

Thirdly, the examiner would like to clarify the teachings of the Martinet et al primary reference. Martinet et al **did not teach or contemplate specifically the expression of a secreted *T. reesei* α-1,2-mannosidase for modification of the protein glycosylation pathway in *Pichia pastoris***, but rather **a detection of full-length *T. reesei* α-1,2-mannosidase in the culture medium was thought to be due to the failure of the potential membrane anchor of the full-length *T. reesei* α-1,2-mannosidase was not functional in *P. pastoris*** (see page 1174, col. 2). Martinet et al stated "one may assume that oligosaccharides can only be trimmed directly after synthesis, **preferably in the ER**, before elongation and mannose-phosphate derivatization occur" (page 1174, col. 1, bottom of last paragraph continues to first line of col. 2). Nevertheless, both forms of the *T. reesei* α-1,2-mannosidase used resulted in the incomplete removal of mannose residues via incomplete *T. reesei* α-1,2-mannosidase digestion **as evidenced by the lack and/or little formation of Man5GlcNAc2**. As a result, both trimmed products and hyperglycosylation products were obtained for the fusion chimeric *T. reesei* α-1,2-mannosidase form. However, Martinet et al specifically noted that **hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the**

temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues (page 1176, col. 1).

Fourthly, with respect to the issues of an inactivation of the Mnn4 gene and/or inactivation of the Och1 gene might be detrimental to cell viability and/or weakness for general use in glycan engineering, please refer to the viability and/or characterization of the double and triple *Saccharomyces cerevisiae* mutants taught by Nakanishi-Shindo et al., Chiba et al. as well as the ΔOch1 *Pichia pastoris* taught by JP 8-336387. Moreover, please also note that **the Och1 gene is upstream of the Mnn4 gene in a protein glycosylation pathway in yeasts**, and since various yeast mutants having a disruption or deletion of the Och1 gene are still viable, there is no basis why an ordinary skilled artisan would not reasonably expect that yeast mutants having a disruption or deletion of the Mnn4 gene would also be viable. Furthermore, none of the rejected claims has anything to do with whether the Mnn4 gene should or should not be disrupted.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Amended claims 90, 92-95, 105 and 114 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-2, 4-6, 9 and 14 of U.S. Patent No. 7,252,933.

Although the conflicting claims are not identical, they are not patentably distinct from each other because a methylotrophic yeast strain transformed with a nucleotide sequence coding for the *T.reseei* α-1,2-mannosidase encoded by SEQ ID NO: 14 and a kit comprising the same in issued U.S. Patent No. 7,252,933 encompasses the *Pichia pastoris* strain (claim 2), a methylotrophic yeast strain in which the OCH1 gene has been disrupted (claim 6), the *T.reseei* α-1,2-mannosidase is engineered to contain the peptide HDEL (claims 4-5) and the expression of *T.reseei* α-1,2-mannosidase is directed by a promoter of a gene selected from the group consisting of AOXI, an AOXII, GAP, YPT1 and FLD, would also fall within the scope of claims 90, 92-95 and 105 of the present application.

Amended claims 90, 92-95, 105 and 114 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 7,507,573.

Although the conflicting claims are not identical, they are not patentably distinct from each other because a genetically engineered *Pichia* strain, wherein said strain is engineered to express (1) a *Trischoderma reesei* α-1,2-mannosidase or a functional

part thereof, (2) an N-acetylglucosaminyltransferase I (GnTI) or a functional part thereof, and (3) a beta-1,4-galactosyltransferase (GalT) or a functional part thereof, and the genomic OCH1 gene of said strain is disrupted in the issued U.S. Patent No. 7,507,573 anticipates the claimed genus of a genetically engineered strain of *Pichia* and a kit comprising the same in the application being examined and, therefore, a patent to the genus would, necessarily, extend the rights of the species or sub- should the genus issue as a patent after the species of sub-genus.

Amended claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225.

The claims of the present application differ from the claims of the issued US Patent 6,803,225 in reciting specifically a genetically engineered *Pichia* yeast strain expressing *T. reesei* α-1,2-mannosidase, and a recited Markush group of specific promoters used to express *T. reesei* α-1,2-mannosidase and/or glucosidase II.

The claims of the present application can not be considered to be patentably distinct over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225 when there is a specific disclosed embodiment of the issued US patent that teaches the use of vectors coding for *T. reesei* α-1,2-mannosidase and its expression under the control of at least AOX1 promoter (see all the examples). Accordingly, the claims of the issued US patent fall within the scope of claims 90, 92-95, 97-107, 109-112 and 114-115 of the present application.

This is because it would have been obvious to an ordinary skilled artisan to modify the claims of the issued US patent by also using vectors coding for *T. reesei* α-1,2-mannosidase and its expression under the control of at least AOX1 promoter for making and using the genetically engineered *Pichia* yeast strain, that support the instant claims. An ordinary skilled artisan would have been motivated to do this because this embodiment is explicitly disclosed or taught in the issued US patent as a preferred embodiment.

In the amendment filed on 3/16/09, Applicants indicate that the non-statutory double patenting rejections will be addressed once the claims are found allowable.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent

Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

/QUANG NGUYEN/

Primary Examiner, Art Unit 1633